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To evaluate the restorative capacity of a contaminated estuarine ecosystem (NY/NJ Harbor Estuary) and to determine the contributions of anaerobic microorganisms in the degradation of refractory fuel components such as polyaromatic hydrocarbons (PAHs) and alkanes in the anoxic harbor sediment; Instrinsic biodegradation of a representative PAH, naphthalene occurs in anaerobic sediment microbial communities. This activity *in-situ* is complicated by other moderators which may be found in the harbor such as toxic heavy metals and/or other contaminants. In addition, the data clearly shows that hydrocarbons such as PAH and alkanes which have been considered to be resistant to biodegradation in the absence of oxygen can indeed be microbially metabolized and mineralized to carbon dioxide. Results indicate that the sulfate reducing anaerobic microorganisms appear to have novel mechanisms for PAH and alkane transformations. In the case of PAH, carboxylation occurs first, then ring reduction occurs prior to ring fission; while in the case of the alkanes, 2 different initial mechanisms of attack have been documented. Anaerobic sediment microbial communities from an impacted harbor estuary have an intrinsic restorative capacity with respect to PAH degradation. In addition, anaerobic microorganisms have novel mechanisms for PAH and alkane transformation. These microbial mechanisms, to our knowledge, have not been previously reported and have implications for biocatalysis as well as for biodegradation. This information potentially can lead to strategies for targeted *in-situ* remediation approaches and to alternatives for managing harbor sediments

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FINAL REPORT

GRANT NUMBER: N00014-93-1-1008

PRINCIPAL INVESTIGATOR: Lily Y. Young

INSTITUTION: Rutgers University

GRANT TITLE: Anaerobic Microbial Degradation of Recalcitrant Fuel Components by Estuarine Sediment Communities

AWARD PERIOD: 1 July 1996 to 30 September, 1998

OBJECTIVE: To evaluate the restorative capacity of a contaminated estuarine ecosystem (NY/NJ Harbor Estuary) and to determine the contributions of anaerobic microorganisms in the degradation of refractory fuel components such as polyaromatic hydrocarbons (PAHs) and alkanes in the anoxic harbor sediment.

APPROACH: For field studies, anaerobic microcosms amended with ^{14}C-naphthalene and other aromatic substrates were subjected to various treatments and monitored for radiolabeled mineralization products. For laboratory studies, selective enrichments were established in defined media for alkanes and various PAHs under four reducing conditions. Analyses included GC and GC/MS of unlabeled and stable isotope labeled compounds.

ACCOMPLISHMENTS: Field microcosm studies indicate that for the bicyclic compound naphthalene (NAP), mineralization varied from 30 to 90% of added substrate in sediment samples which had previous long-term PAH exposure. As expected, sediments from non-contaminated sites showed no measurable activity within 350 days. The degradation of ^{14}C-NAP resulted in the accumulation of $^{14}CO_2$ with no production of $^{14}CH_4$. No significant difference in activity or rate was observed between unamended cultures and those amended with select nutrients and electron acceptors, suggesting that activity was not nutrient limited. Sediment dilution experiments showed enhancement of overall anaerobic activity as well as PAH mineralization by up to an order of magnitude. Additionally, when the sediment microcosms were amended with an active NAP culture derived from the same site, we observed a significant increase in the NAP mineralization rate. In inoculated microcosms, 60% of added ^{14}C-NAP was mineralized within ten days, as compared to uninoculated microcosms in which it took 100 days.

Selective enrichments on PAH compounds yielded highly active and readily propagated cultures on NAP, 2-methylnaphthalene (2-MNAP) and phenanthrene (PHE), while no activity was seen with pyrene. Mineralization of radiolabeled substrates was documented and activity was dependent on sulfate reduction. Use of stable isotopes and analyses by GC/MS determined that for all 3 PAHs, carboxylation was an initial key reaction. In further studies on the anaerobic NAP pathway, results indicate that after the initial carboxylation of NAP to 2-naphthoic acid (2-NA) sequential hydrogenation to decalin-2-NAs occurred through 5 steps with each step eliminating one double bond. We believe that this is the first clear demonstration of an anaerobic mechanism for PAH degradation.

Laboratory investigations with harbor sediment have also resulted in the isolation of a pure culture capable of anaerobic alkane degradation. It is a Gram-negative, sulfate reducing rod, designated AK01. Physiological, biochemical and 16S rRNA gene sequence characterization indicate that it is in the delta division of the class Proteobacteria and related

to the genus *Desulfosarcina.* Alkane oxidation ability of AK01 was compared to that of one of the few other known anaerobic alkane degraders, strain Hxd3 (formerly known as*Desulfobacterium oleovorans*). Recovery of deuterated fatty acids formed upon degradation of perdeuterated pentadecane confirmed that the alkane was oxidized to fatty acids by the two strains. Additionally, differences in cellular fatty acid compositions in the two strains implies the existence of two distinct mechanisms of alkane oxidation.

Evidence indicates that in strain AK01, when C-even alkanes are used as substrate, the predominant cellular fatty acids are C-even, and C-odd alkanes yielded C-odd fatty acids. For example, biodegradation of hexadecane by AK-01 yielded 97% of the total cellular fatty acids as C-14, C-16, C-18 and C-20 fatty acids. When heptadecane was used, 91% of the total cellular fatty acids were recovered as C-13, C-15, C-17, C-19 and C-21 fatty acids. In constrast, biodegradation of alkanes by Hxd3 generated opposite results. For example, with growth on pentadecane 95% of the total cellular fatty acids were C-even (mostly C-14, C-16 and C-18); while growth on hexadecane yielded 89% total cellular fatty acids as mostly as C-13, C15 and C-17. Detailed work in GC/MS data on fatty acids formed by each strain has generated evidence of 2 novel pathways of anaerobic alkane degradation.

CONCLUSIONS: Instrinsic biodegradation of a representative PAH, naphthalene occurs in anaerobic sediment microbial communities. This activity *in-situ* is complicated by other moderators which may be found in the harbor such as toxic heavy metals and/or other contaminants. In addition, the data clearly shows that hydrocarbons such as PAH and alkanes which have been considered to be resistant to biodegradation in the absence of oxygen can indeed be microbially metabolized and mineralized to carbon dioxide. Results indicate that the sulfate reducing anaerobic microorganisms appear to have novel mechanisms for PAH and alkane transformations. In the case of PAH, carboxylation occurs first, then ring reduction occurs prior to ring fission; while in the case of the alkanes, 2 different initial mechanisms of attack have been documented.

SIGNIFICANCE: Anaerobic sediment microbial communities from an impacted harbor estuary have an intrinsic restorative capacity with respect to PAH degradation. In addition, anaerobic microorganisms have novel mechanisms for PAH and alkane transformation. These microbial mechanisms, to our knowledge, have not been previously reported and have implications for biocatalysis as well as for biodegradation. This information potentially can lead to strategies for targeted *in-situ* remediation approaches and to alternatives for managing harbor sediments.

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